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Genetic mapping of *hph2*, a mutation affecting amino acid transport in the mouse

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Abstract. We describe the genetic mapping of hyperphenylalaninemia 2 (*hph2*), a recessive mutation in the mouse that causes deficient amino acid transport similar to Hartnup Disorder, a human genetic amino acid transport disorder. The *hph2* locus was mapped in three separate crosses to identify candidate genes for *hph2* and a region of homology in the human genome where we propose the Hartnup Disorder gene might lie. The mutation maps to mouse Chromosome (Chr) 7 distal of the simple sequence length polymorphism (SSLP) marker *D7Mit140* and does not recombine with *D7Nds4*, an SSLP marker in the fibroblast growth factor 3 (*Fgf3*) gene. Unexpectedly, the mutant chromosome affects recombination frequency in the *D7Mit12* to *D7Nds4* interval.

Introduction

Several recessive, N-ethyl-N-nitrosourea-induced mutations in the mouse affecting phenylalanine catabolism have previously been isolated and analyzed (McDonald and Bode 1988; McDonald et al. 1990; Shedlovsky et al. 1993). Another mutation, *hph2*, isolated in the same screen affects amino acid transport rather than phenylalanine catabolism. The mutant phenotype (Symula et al. 1996) resembles Hartnup Disorder (Baron et al. 1956; Levy 1995). Here we describe the genetic mapping of the *hph2* mutation to mouse distal Chr 7. Analysis of these data revealed that the *hph2* mutant chromosome unexpectedly affected recombination in the region when inherited in one mating orientation. This genetic phenomenon deserves further investigation.

Materials and methods

Animals. Mice were bred and maintained by standard methods of mouse husbandry (Les 1966). SWR/J (SWR) and C57BL/6J (B6) mice were obtained from The Jackson Laboratory (Bar Harbor, Me.); BTBR/Pas (BTBR) and 129/Sv/Pas-Sl/+ (129) were bred in our laboratory. All mice were bred and maintained on Purina 5001 feed (Purina Mills, Richmond, Ind.). HPH2 mice were congenic on BTBR (N = 13). The HPH2 phenotype was determined by delayed clearance of an injected load of phenylalanine as described by McDonald and associates (1990). Mutants were distinguished from heterozygotes by elevated (≥5-fold) blood phenylalanine 2 h after challenge.

DNA isolation. DNA was prepared by a scaled-down modification of Phillips and Nadeau (1984).

PCR. Amplification of SSLP markers was performed in buffer containing: 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.05 mM dNTPs, 0.0924 μM each primer, 0.25 unit Taq DNA polymerase (Promega, Madison, WI) and 1.5 μl DNA per 12.5 μl reaction. Amplification was performed in a MJ Research thermocycler (Watertown, Mass.) with the following profile: 94°C for 3 min, 30 cycles of (94°C for 15 s., 55°C for 2 min, 72°C for 2 min), 72°C for 7 min, 15°C for 1 min. Amplimers were resolved on agarose gels (3–4%) and visualized by ethidium bromide staining.

The SSLP markers employed are *D7Mit7, D7Mit12, D7Mit71, D7Mit140, D7Mit141, D7Mit189, D7Mit223, D7Mit293, D7Mit362,* and *D7Nds4.* These will be referred to by the MIT number, e.g., *D7Mit12* will be indicated as *12.* Each marker has been mapped to distal Chr 7 (see Dietrich et al. 1994 and Mouse Genome Database 1996). Allele sizes for BTBR and 129/Sv Pas have been entered in the MIT database.

SSLP allele sizing. Each PCR amplification reaction was spiked with a small amount of alpha-^{32}P dCTP (Dupont-NEN, Boston, Mass.). Amplimers were resolved on 8% denaturing polyacrylamide gels (BioRad, Hercules, Calif.) and visualized by autoradiography with Fuji Medical X-Ray film (Fuji Film Co, Japan). BTBR and 129 amplimers were sized with a sequencing ladder run in adjacent lanes. In addition, amplimers from B6, DBA/2J, and AKR/J or A/J were used as standards.

Calculation of genetic distance. Recombination frequency between adjacent markers was converted to genetic distance in cM, assuming no double recombinants.

Results

Mapping hph2. The *hph2* mutation, induced in the inbred BTBR mouse strain, was identified in mice of mixed BTBR and 129 genetic background. In an initial mapping cross, homozygous mutant males from this outbred stock were mated to *M. spretus,* and F_1 daughters were backcrossed to their homozygous mutant fathers. Twenty-four progeny were tested for mutant phenotype, and DNA was prepared from tissues of each animal. The approximate map position of *hph2* was determined by genome scanning with SSLP markers polymorphic between the two laboratory inbred strains and *M. spretus.* Each marker could be employed to scan 22 cM at 95% confidence (Silver and Buckler 1986), and approximately 95% of the genome was scanned (data not shown). The mutation mapped to Chr 7 and cosegregated with markers *41, 12,* and *Nds4* at map positions 60, 66, and 72 cM from the centromere, respectively (Mouse Genome Database 1996).

To confirm and refine the map position, two larger crosses were constructed with HPH2 mutant mice congenic with BTBR. Several SSLP markers on distal Chr 7 were polymorphic between BTBR versus strains SWR and B6 (Dietrich et al. 1994). Therefore, the latter two strains were chosen for large mapping crosses that were carried out both by intercrossing F_1 animals to produce an F_2 generation and by backcrossing to the BTBR mutant line. BTBR-*hph2/hph2* females breed poorly; therefore, F_1 males were

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backcrossed to BTBR-*hph2*/ + females, and F_1 females were backcrossed to BTBR-*hph2/hph2* males. Mutant phenotypes were determined by delayed phenylalanine clearance (McDonald et al. 1990). From the F_1 intercross, progeny were first screened for mutant phenotype. Among the non-mutant class only heterozygotes were further screened. These were distinguished from non-carriers by their genotype at the linked marker *71*. From the SWR crosses, 453 progeny were obtained, and DNA from each was screened for recombination events distal to marker *7*, 53 cM from the centromere. Initially, animals were genotyped for *7* and *Nds4*, one of the distal markers in these crosses (Dietrich et al. 1996). Recombinants in this interval were then genotyped for a number of additional markers in the region. It became clear after scoring only 18 animals from the SWR crosses that *hph2* mapped distal to marker *71*, which was then used for all subsequent recombinant screening. Next, recombinants in the *71* to *Nds4* interval (52 total) were genotyped for *12;* recombinants distal to *12* (19 animals) were then analyzed for marker *140*. The number of recombinants in each interval is shown in Figure 1a. Among animals recombinant between *140* and *Nds4*, markers *189*, *223*, *293*, and *362* all cosegregated with *Nds4*. Similarly, from the B6 cross, 107 animals were first screened for recombination between *12* and *Nds4*. Thirteen recombinants were identified (see Fig. 1b) and genotyped for *141*, *189*, and *293* (*140* was not polymorphic in this cross). All of these markers cosegregated with *Nds4*.

In summary, the *hph2* mutation mapped to distal Chr 7 in both the B6 and the SWR crosses, confirming the results of the *M. spretus* backcross. Further, the mutation did not recombine with *Nds4*. In the crosses with SWR, *hph2* mapped distal to marker *140*. No double recombinants between markers *71* and *Nds4* were observed among approximately 200 animals in the SWR crosses (Fig. 1 and data not shown). These loci are presented in a linkage map of distal Chr 7 along with distances in cM from the centromere (Fig. 2).

Orientation of F_1 mating affects map distance between 12 and Nds4. In the crosses with SWR it was observed that the frequency of recombination in the *12* to *Nds4* region depended upon the parental orientation of the F_1 hybrid animals. This phenomenon is illustrated in Table 1. In total, eight recombinants between *12* and *Nds4* were recovered among 227 progeny of F_1 females of the SWR backcross. Strikingly, six of these recombinants were recovered among the 53 progeny of (BTBR × SWR) *hph2*/ + F_1s, and only two among the 174 progeny of (SWR/J × BTBR) *hph2*/ + F_1s.

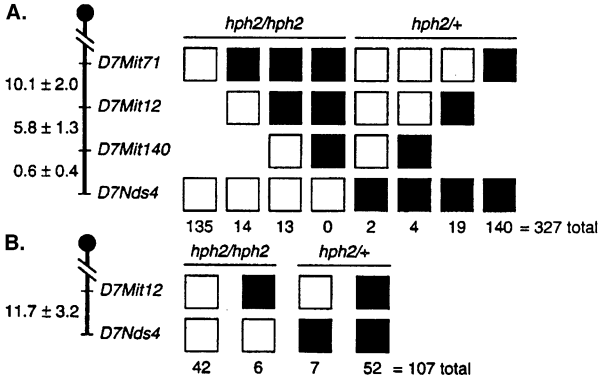

Fig. 1. Chromosomes recovered in the SWR (**a**) and B6 (**b**) crosses and map position of *hph2*. Marker names are at the left along with distances, in cM, between them, phenotypes are above, and the numbers recovered for each class are beneath the chromosomes. Filled boxes indicate heterozygosity for BTBR and SWR or B6 for that marker, open boxes homozygosity for BTBR. Only genotypes of animals displaying mutant phenotypes or those known to be mutant carriers owing to heterozygosity at the linked markers are presented.

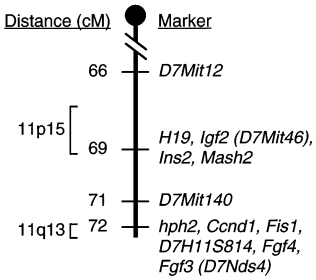

Fig. 2. A linkage map of the *hph2* region of distal Chr 7, with positions taken from the Mouse Genome Database, 1996. The position of *hph2* is shown relative to genes mapped to this region. Regions of the human genome that are homologous to mouse distal Chr 7 are indicated by the brackets at the left of the chromosome (see Debry and Seldin 1996). Note that *hph2* may be proximal or distal to *D7Nds4*. No markers have been reliably mapped distal to *D7Nds4* except *Fgf4* and *Ccnd1*, which lie within several hundred kb of this marker.

Table 1. Summary of recombination frequencies in the *D7Mit12* to *D7Nds4* interval in the mapping crosses and crosses between wildtype BTBR and SWR.

Cross	Recombinants/total meioses
(SWR × BTBR) F_1 *hph2*/+ × BTBR-*hph2/hph2*	2/174
(BTBR × SWR) F_1 *hph2*/+ × BTBR-*hph2/hph2*	6/53
(SWR × BTBR) F_1 +/+ × BTBR-+/+	3/46
(BTBR × SWR) F_1 +/+ × BTBR-+/+	3/41

To determine whether this difference required the mutant chromosome or was a property of the two wild-type strains, crosses between non-mutant BTBR and SWR were performed. In the absence of the *hph2* mutation, no differences in recombination frequency were observed.

The effect on recombination in F_1 females (not males) that inherited the *hph2* mutation from their fathers suggested an effect of maternal imprinting (see Barlow 1994). Therefore, the mapping crosses and the BTBR-*hph2/hph2* pedigree records were reviewed for evidence of skewing of the phenotypic ratios, another indication of imprinting. The phenotypic ratios of mutant to non-mutant were consistent with those expected from standard Mendelian inheritance (Table 2, lines 1–5 and 7). We note, however, the dramatic excess of mutants in Table 2, line 6. This observation must be pursued separately.

Discussion

Mapping hph2. The *hph2* mutation was mapped to mouse distal Chr 7, 0.6 cM distal of marker *140*. In approximately 600 meioses from three different crosses, *hph2* did not recombine with *Nds4*, a polymorphism in the fibroblast growth factor 3 (*Fgf3*) gene, nor with any other SSLP marker distal of *140* (Fig. 2 and data not shown). Thus, *hph2* lies within 0.8 cM of *Fgf3* (either proximal or distal) with 90% confidence. Determining the *hph2* map position is an important step in the genetic characterization of the mutation. The *hph2* map position also provides a candidate region in the human genome (see below) against which Hartnup Disorder can be mapped and allows explicit testing for genetic heterogeneity in human pedigrees. It seems that more than one gene must be involved in this condition, since among affected Hartnup Disorder siblings the niacin deficiency is variable (Scriver 1988). Identification of such additional loci must await further study.

Several human genes have been identified that are involved in amino acid transport. Among those that are unmapped, none has the physiological activity associated with *hph2*, and thus they are not good candidates for this gene. Two others involved in amino

Table 2. Phenotypic ratios of progeny recovered in the mapping crosses and in maintenance crosses of *hph2* on the BTBR background. Phenotype was determined by phenylalanine clearance.

Cross	Mutant	Non-mutant
BTBR-*hph2*/+ × BTBR-*hph2/hph2*	125	134
BTBR-*hph2*/+ × BTBR-*hph2*/+	25	66
(SWR × BTBR) F_1 *hph2*/+ × BTBR-*hph2/hph2*	90	84
(BTBR × SWR) F_1 *hph2*/+ × BTBR-*hph2/hph2*	25	28
(SWR × BTBR) F_1 *hph2*/+ × BTBR-*hph2*/+	21	78
BTBR-*hph2*/+ × (SWR × BTBR) F_1 *hph2*/+	22	35
(B6 × BTBR) F_1 *hph2*/+ × BTBR0*hph2/hph2*	17	33

acid transport have been mapped in the human, to Chr 2p (Lee et al. 1993; Pras et al. 1994; Calonge et al. 1994): SLC3A1, which has activity similar to that of *hph2;* and solute carrier family 1, member 4 (SLCIA4), which also stimulates neutral amino acid transport when expressed in *Xenopus* oocytes (Shafqat et al. 1993; Arriza et al. 1993). However, since human Chr 2p has no known homology with mouse distal Chr 7, these two human loci are not good candidates for *hph2*.

Recently Pontoglio and colleagues (1996) have demonstrated that inactivation of the transcriptional activator *Hnf1* results in aminoaciduria, a wasting syndrome, and phenylketonuria. However, this interesting strain differs from the HPH2 strain both in genetic mapping and in response to niacin.

The homologous region of the human genome is Chr 11q13. Like mouse distal Chr 7, this region contains no known loci involved in amino acid transport. Further potential candidates are loci of unknown function in the region such as D11S814, an uncharacterized CpG island distal to FGF4 (Lammie et al. 1992). The location of D11S814 only a few hundred kilobases from FGF3 in both the human and the mouse genomes is consistent with the probable position of *hph2*. Insulin, a potent stimulator of amino acid transport (McGivan and Pastor-Anglada 1994) is encoded by the *Ins2* gene, which maps to distal Chr 7. However, this locus is proximal to *140* (Copeland et al. 1993; Dietrich et al. 1994) and thus is not a candidate gene. Two other growth factors, *Fgf3* and *Fgf4*, are potential candidates in view of their tight linkage to *hph2* and the stimulatory effect of fibroblast growth factor on amino acid transport in 3T3 cells (Quinlan and Hochstadt 1977). Fine structure mapping or molecular analysis of *Fgf3* and *Fgf4* will be required to determine whether *hph2* affects any of these genes.

Finally, candidates may also be identified in the extensive physical maps (Julier et al. 1990; Litt et al. 1993) and expressed sequence-tagged (EST) maps (Rosier et al. 1995) being constructed on Chr 11. Genes involved in sodium-dependent transport of glucose, neurotransmitters, and amino acids generally code for proteins with approximately 12 transmembrane domains (Reizer et al. 1994; Saier 1994). Any gene with a predicted structure of this kind would be an attractive candidate for Hartnup Disorder. First, the region of human Chr 11 should be tested as a candidate for the recessive determinant of Hartnup's Disorder by homozygosity mapping (Krugylak et al. 1995). Ultimately, gene identification can be confirmed by sequence comparison of the mouse homolog between HPH2 and wild-type BTBR mice.

Effect of parental origin on genetic distance. The parental origin of the *hph2* chromosome in the SWR crosses (but not the B6 crosses) dramatically influenced the frequency of recombination in F_1 females. Interestingly, it has been shown that the distal region of Chr 7 encodes genes that are imprinted (Cattanach and Beechey 1985; Searle and Beechey 1990). Paternal imprinting (maternal expression) of *hph2* would result in a ratio of mutant to non-mutant progeny of 1:1 in *hph2*/ + × *hph2*/ + crosses, rather than the expected 1:3. By contrast, maternal imprinting (paternal expression) would have resulted in only mutant progeny from the cross *hph2*/ + × *hph2/hph2,* instead of a 1:1 ratio of mutant to non-mutant. No such indications of imprinting were observed from the ratio of mutant to non-mutant progeny of crosses involving *hph2.*

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References

Arriza JL, Kavanaugh MP, Fairman, WA, Wu Y-N, Murdoch GH, North RA, Amara SG (1993) Cloning and expression of a human neutral amino acid transporter with structural similarity to the glutamate transporter gene family. J Biol Chem 268, 15329–15332

Barlow DP (1994) Imprinting: a gamete's point of view. Trends Genet 10, 194–199

Baron DN, Dent CE, Harris H, Hart EW, Jepson JB (1956) Hereditary pellagra-like skin rash with temporary cerebellar ataxia, constant renal amino-aciduria, and other bizarre biochemical features. Lancet 2, 421–428

Calonge MJ, Gasparini P, Chillaron J, Gallucci M, Rousaud F, Zelante L, Testar X, Dallapiccola B, DiSilverio F, Barcelo P, Estivill X, Zorzano A, Nunes V, Palacin M (1994) Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. Nature Genet 6, 420–425

Cattanach BM, Beechey CV (1985) Differential activity of maternally and paternally derived chromosome regions in mice. Nature 315, 496–498

Copeland NG, Gilbert DJ, Jenkins NA, Nadeau JH, Eppig JT, Maltais LJ, Miller JC, Dietrich WF, Steen RG, Lincoln SE, Weaver A, Joyce DC, Merchant M, Wessel M, Katz H, Stein LD, Reeve MP, Daly MJ, Dredge RD, Marquis A, Goodman N, Lander ES (1993) Genome Maps IV. Science 262, 67

Debry RW, Seldin MF (1996) Human/Mouse Homology Relationships, National Center for Biotechnology Information. World Wide Web (URL: http://www3.ncbi.nlm.nih.gov/Homology/)

Dietrich WF, Miller JC, Steen RG, Merchant M, Damron D, Nahf R, Gross A, Joyce DC, Wessel M, Dredge RD, Marquis A, Stein LD, Goodman N, Page DC, Lander ES (1994) A genetic map of the mouse with 4006 simple sequence length polymorphisms. Nature Genet 7, 220–245 Supplemented by additional markers in: Whitehead Institute/MIT Center for Genome Research, Genetic Map of the Mouse, Database (http://www-genome.wi.mit.edu) Release 10, June 1996

Dietrich WF, Miller J, Steen R, Merchant MA, Damron-Boles D, Husain Z, Dredge R, Daly MJ, Ingalls KA, O'Conner TJ, Evans CA, DeAngelis MM, Levinson DM, Kruglyak L, Goodman N, Copeland NG, Jenkins NA, Hawkins TL, Stein L, Page DC, Lander ES (1996) A comprehensive genetic map of the mouse genome. Nature 380, 149–152

Julier C, Nakamura Y, Lathrop M, O'Connell P, Leppert M, Litt M, Mohandas T, Lalouel J-M, White R (1990) A detailed genetic map of the long arm of chromosome 11. Genomics 7, 335–345

Krugylak L, Daly MJ, Lander ES (1995) Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. Am J Hum Genet 56, 519–527

Lammie GA, Smith R, Silver J, Brookes S, Dickson C, Peters G (1992) Proviral insertions near the cyclin D1 gene in mouse lymphomas: a parallel for BCL1 translocations in human B-cell neoplasms. Oncogene 7, 2381–2387

Lee W-S, Wells RB, Sabbag RV, Mohandas TK, Hediger MA (1993) Cloning and chromosomal localization of a human kidney cDNA involved in cystine, dibasic, and neutral amino acid transport. J Clin Invest 91, 1959–1963

Les EP (1996) Husbandry. In *Biology of the Laboratory Mouse*, EL Green, ed (New York: McGraw-Hill), pp 29–37

Levy HL (1995) Hartnup Disorder. In *Metabolic and Molecular Bases of Inherited Disease*, CR Scriver, AL Beaudet, WS Sly, D Valle, eds (New York: McGraw-Hill), pp 3629–3642

Litt M, Kramer P, Hauge XY, Weber JL, Wang Z, Wilkie PJ, Holt MS, Mishra S, Donis-Keller H, Warnich L, Retief AE, Jones C, Weissenbach J (1993) A microsatellite-based index map of human chromosome 11. Hum Mol Genet 2, 909–913

McDonald JD, Bode VC (1988) Hyperphenylalaninemia in the *hph-1* mouse mutant. Pediatr Res 23 63–67

McDonald JD, Bode VC, Dove WF, Shedlovsky A (1990) *Pah*$^{hph\text{-}5}$*:* a

mouse mutant deficient in phenylalanine hydroxylase. Proc Natl Acad Sci USA 87, 1965–1967

McGivan JD, Pastor-Anglada M (1994) Regulatory and molecular aspects of mammalian amino acid transport. Biochem J 299, 321–334

Mouse Genome Database (MGD) 3.1, Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine. World Wide Web (URL: http:/www.informatics.jax.org/). (February 1996)

Phillips S, Nadeau J (1984) Mouse News Letter 70, 83

Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C (1996). Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria and renal Fanconi syndrome. Cell 84, 575–585

Pras E, Arber N, Aksentijevich I, Katz G, Schapiro JM, Prosen L, Gruberg L, Harel D, Liberman U, Weissenbach J, Pras M, Kastner DL (1994) Localization of a gene causing cystinuria to chromosome 2p. Nature Genet 6, 415–419

Quinlan DC, Hochstadt J (1977) The regulation by fibroblast growth factor of early transport changes in quiescent 3T3 cells. J Cell Physiol 93, 237–246

Reizer J, Reizer A, Saier MH (1994) A functional superfamily of sodium/solute symporters. Biochim Biophys Acta 1197, 133–166

Rosier M-F, Reguigne-Arnould I, Coullin P, Devignes M-D, Auffray C (1995) Regional assignment of 68 new human gene transcripts on chromosome 11. Genome Res 5, 60–70

Saier MH (1994) Convergence and divergence in the evolution of transport proteins. BioEssays 16, 23–29

Scriver CR (1988) Nutrient-gene interactions: the gene is not the disease and vice-versa. Am J Clin Nutr 48, 1505–1509

Searle AG, Beechey CV (1990) Genome imprinting phenomena on mouse chromosome 7. Genet Res 56, 237–244

Shafqat S, Tamarappoo BK, Kilberg MS, Puranam RS, McNamara JO, Guadano-Ferraz A, Fremeau RT (1993) Cloning and expression of a novel Na^+-dependent neutral amino acid transporter structurally related to mammalian Na^+/glutamate cotransporters. J Biol Chem 268, 15351–15355

Shedlovsky A, McDonald JD, Symula D, Dove WF (1993) Mouse models of human phenylketonuria. Genetics 134, 1205–1210

Silver J, Buckler CE (1986) Statistical considerations for linkage analysis using recombinant inbred strains and backcrosses. Proc Natl Acad Sci USA 83, 1423–1427

Symula DJ, Shedlovsky A, Guillery EN, Dove WF (1996) A candidate mouse model for Hartnup disorder deficient in neutral amino acid transport. Mamm Genome 8, 102–107